A Partial Characterization of an Autolytically Solubilized Cell Wall Glucan

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ABSTRACT

Incubation of purified cell wall fragments from corn (Zea mays) coleoptiles results in solubilization of some of the wall dry matter. The portion of the weight loss due to enzymatic autolysis is due mainly to solubilization of a glucan and, to a small extent, to liberation of free glucose. No other carbohydrate wall components or sugars other than glucose are solubilized despite the high concentrations of, for example, galactans, arabans, and xylans in the walls. The glucan has been partially characterized and found to be a lichenan-like polymer composed of 1→3 and 1→4 linked glucosyl units.

A previous study from this laboratory (13) was concerned with methods for the isolation of pure cell wall fragments from corn coleoptile tissue. The cell wall preparations obtained appeared free of particulate contamination in the light microscope (13) and similarly prepared walls from other tissues seemed homogeneous when examined in the electron microscope (12). Reisolation of the walls, after deliberate contamination with a cytoplasmic enzyme, indicated that the preparative procedure used was adequate to remove the added cytoplasmic contamination (10, 14). Thus, within the limits of certainty characteristics of attempts to localize cellular activities, studies of such preparations yield information on a wall-localized enzyme activities that could play a role in wall synthesis and hydrolysis. Subsequently, it was demonstrated that such wall preparations would autolyze as evidenced by a loss in dry weight upon incubation in water (15–17). In this paper evidence is presented to indicate that wall-localized enzymes solubilize only one of the polysaccharides of the wall. This polysaccharide resembles, in terms of its hydrolysis products, the glucans, lichenan (32) and oat and barley glucan (31).

MATERIALS AND METHODS

Cell Wall Preparation and Incubation. Cell walls were prepared from 5-day corn coleoptile tissue by homogenization in glycerol and filtration through a glass bead filter, as previously described (13). Glycerol was removed, prior to incubation, by washing the walls with 95% ethyl alcohol, then acetone, and then ether, all at −10 to −20 C (17).

Solvent-washed cell wall powders were stored at −20 C over anhydrous calcium sulfate (17) and were stable with regard to autolytic capability for at least 1 year. Incubation was at 37 C in water with a wall concentration of 10 mg/ml and with occasional resuspension by shaking. “Zero” time controls were obtained by heating the suspended walls in a boiling water bath immediately after suspension. All incubations were terminated at the indicated time in the same way as the zero time controls. Residual wall was collected by filtration through Millipore filters (0.45 μm) or through washed and tared discs of Whatman No. 540. The collected walls were washed with water (3 × 1 ml) and the wash waters were combined and filtrate was frozen and thawed three times to promote retrogradation of solubilized wall components and these then were collected by filtration and weighed. Unless otherwise specified, reagents were of analytical quality and were used without further purification. Glycerol was distilled in vacuo prior to use. Celllobiose and gentiobiose were obtained from Nutritional Biochemicals. A sample of laminariaribiose was obtained from Professor W. Z. Hassid. Later, samples of laminariaribiose were prepared by partial acid hydrolysis of laminaran (Pfalz and Bauer, Inc.) and the di- and trisaccharides were isolated by chromatography on Sephadex G-10 or G-15. The trisaccharide 3-O-β-cellubioxyb-d-glucose was obtained from Dr. E. T. Reese. Lichenan was obtained from Pierce Chemical Co. and was purified as the acetate (42). A sample of oat glucan prepared by Dr. A. Perlin and a sample of lichenan [5220] prepared by Dr. D. Manners were obtained from Dr. E. T. Reese. Bacterial amylase was from Novo Industri A/S Copenhagen, Denmark.

Paper Chromatography. Descending chromatography was employed on Whatman No. 1 paper with pyridine-ethyl acetate-water (2:8:1) as the irrigating solvent (40). The ends of the paper were serrated to permit the solvent to drip easily off the paper in those cases where prolonged irrigation was required (37). Sugars were located with alkaline silver nitrate (40), and authentic standards were compared with hydrolysis products on the same paper sheet.

Gas-Liquid Chromatography. Analyses were conducted with an F and M model 402 chromatograph with a hydrogen flame ion detector. The column types used are indicated in the legends and include 2% OV-1, ⅛ inch × 6 feet on Gas-Chrom Z and 3% SE-30 on Supelcoport available from Applied Science Laboratories, State College, Pa., and Supelco, Inc., Bellefonte, Pennsylvania.

1 Abbreviations: celllobiose: 4-O-β-D-glucopyranosyl glucose; gentiobiose: 6-O-β-D-glucopyranosyl-4-O-β-D-glucopyranose; laminaribiose: 3-O-β-D-glucopyranosyl-4-O-β-D-glucopyranose; laminaran: 3-O-β-D-glucopyranosyl glucose; 3-O-β-D-cellubiosyl-D-glucose; O-β-D-glucopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→3)-β-D-glucopyranose; GLC: gas-liquid chromatography; TLC: thin layer chromatography.
RESULTS

The time course of wall weight loss during incubation in water at 37 C is shown in Figure 1. Buffers were not used so as to permit weighing the fractions solubilized and toluene was added for this experiment to prevent microbial growth. The pH was 5.8 before incubation and dropped to 5.2 during incubation. The zero time samples were boiled immediately after suspension of the wall in water. The remaining samples were boiled after incubation for the indicated time.

Nonenzymatically Solubilized Components. The nonenzymatic, zero time, solubilization of wall components during boiling of the wall and washing of the wall residue with water amounts to about 11 mg per 100 mg of wall weight. About 9 mg of the 10-mg loss was accounted for as glycerol by GLC (Table I) and TLC analysis (Table II). The glycerol is presumably a residual contaminant of the glycerol used in wall preparation. About 1 mg of the 10-mg loss is due to glucose as identified by GLC (Table I) and TLC (Table II) and glucose oxidase. The amount of glucose was variable, ranging from 0.1 to 1% of the wall weight. It may represent free glucose, or some easily degradable compound, or enzymatic action that occurs during wall suspension prior to boiling. The remaining 1 mg (1% of the total wall weight) is unaccounted for except that a portion of this may be water since the walls are hygroscopic. Acid hydrolysis (121 C for 1 hr in 1 m trifluoroacetic acid) of this nonenzymatically liberated, water-soluble portion of the wall does not lead to an increase in reducing sugars so that oligosaccharides are not present. Thus, the water-soluble constituents of glycerol-prepared cell walls consist of residual

![Graph](image)

Fig. 1. Time course of cell wall autolysis. Incubation is in water with added toluene at 37 C for the indicated time. Weight loss was measured after collecting by filtration that portion of the wall which did not become soluble. Glucan was collected and weighed after inducing retrogradation by repeated freezing and thawing of the filtrate. Reducing substance was measured in the filtrate after removal of the glucan. All values are the averages of three closely agreeing replicates. For convenience in plotting the observed weight loss is divided by two.

Table I. Retention Times for Trimethylsilyl Sugar Ethers

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Column</th>
<th>Temperature (°C)</th>
<th>Retention Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerol</td>
<td>OV-1</td>
<td>100</td>
<td>1.3</td>
</tr>
<tr>
<td>Glucose</td>
<td>OV-1</td>
<td>170</td>
<td>1.7, 2.5</td>
</tr>
<tr>
<td>Cellobiose</td>
<td>OV-1</td>
<td>220</td>
<td>4.2, 6.1</td>
</tr>
<tr>
<td>Laminaribiose</td>
<td>SE-30</td>
<td>240</td>
<td>6.0, 8.2</td>
</tr>
<tr>
<td>Gentiobiose</td>
<td>OV-1</td>
<td>220</td>
<td>8.7</td>
</tr>
<tr>
<td>Gentiobiose</td>
<td>SE-30</td>
<td>240</td>
<td>11.2</td>
</tr>
<tr>
<td>Gentiobiose alditol</td>
<td>OV-1</td>
<td>220</td>
<td>7.3</td>
</tr>
<tr>
<td>Laminaribiose</td>
<td>OV-1</td>
<td>220</td>
<td>5.7, 6.4</td>
</tr>
<tr>
<td>Laminaribiose</td>
<td>SE-30</td>
<td>240</td>
<td>7.8, 8.7</td>
</tr>
<tr>
<td>3-O-α-Celllobiosyl-d-glucose</td>
<td>OV-1</td>
<td>270</td>
<td>6.8, 7.9</td>
</tr>
</tbody>
</table>

1 Retention times for sugars are for the α and β anomers, respectively. Silylation procedure and chromatographic conditions are as described in the text.

Table II. Migration of Sugars Relative to Glycerol on a Thin Layer Chromatogram

Relative migration rate of various sugars was determined on thin layer Silica Gel G chromatograms with 1-butanol-acetic acid-ethyl ether-water (9:6:3:1, v/v) as solvent. The actual migration of glycerol on a 20-cm plate was 14.5 and 15 cm after 3 and 4 developments, respectively.

<table>
<thead>
<tr>
<th>Sugar</th>
<th>No. of Developments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerol</td>
<td>1.00</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.92</td>
</tr>
<tr>
<td>Laminaribiose</td>
<td>0.72</td>
</tr>
<tr>
<td>Cellobiose</td>
<td>0.64</td>
</tr>
<tr>
<td>Gentiobiose</td>
<td>0.57</td>
</tr>
<tr>
<td>Laminaritriose</td>
<td>0.47</td>
</tr>
<tr>
<td>3-O-α-Celllobiosyl-d-glucose</td>
<td>0.38</td>
</tr>
</tbody>
</table>
aliquots of cose produced within preparation during this ration. Tween yields from period theing appears. The data of experiment of Fig. 1, the autolytic weight loss during the first 24 hr, corrected for zero time weight loss, amounts to 6.8 mg (17.8 - 11.0) per 100 mg of wall. Retrogradable polyglucan accounts for 3.6 mg of this loss and reducing substances, calculated as glucose (see Fig. 2), amount to 1.8 mg. The remainder is a polyglucan which does not retrograde but which yields only glucose upon strong acid hydrolysis and is apparently identical to the retrogradable glucan in terms of the products formed upon weak acid hydrolysis.

No appreciable further weight loss occurs after 24 hr at 37 C, the reaction being 90% complete in 8 hr. A slow continuous increase of reducing substance does occur during the period between 24 and 72 hr and this is accounted for by a decrease of polyglucan. Between 24 and 48 hr the changes are 0.53 mg of glucan disappearing from the incubation solution and 0.51 mg of reducing substance appearing. Between 48 and 72 hr, 0.49 mg of glucan is lost and 0.28 mg of reducing substance appears.

The data of Figure 2 show the process of wall autolysis during the first 8 hr. The range of variability of glucan production from different wall preparations is illustrated by comparison of glucan yields from preparations XIV and XV. The yield is between 4 and 7% of the wall weight. Unpublished studies (A. Murray) suggest that the variability between different wall preparations may be owing to variable inactivation of wall enzymes during the solvent treatments employed in wall preparation. Replicate incubations from a single wall preparation, preparation XV in this case, show little variability, agreeing within 0.5%. The ratio of anhydroglucose solubilized to glucose produced varies during the course of wall autolysis, in this case being 8 to 1 during the 1- to 4-hr period and 4 to 1 during the 4- to 8-hr interval. Glucose accounts for most of the

![Fig. 2. Time course of wall autolysis. Conditions were as described for Figure 1. Glucose was measured by glucose oxidase on aliquots of the filtrate obtained from the incubated walls.](image)

**Fig. 2.** Time course of wall autolysis. Conditions were as described for Figure 1. Glucose was measured by glucose oxidase on aliquots of the filtrate obtained from the incubated walls.

Fig. 3. Recovery of reducing sugar and glucose from glucan hydrolyzed for the indicated time in 1 M trifluoroacetic acid at 121 C. Conditions and assay were as described in the text.溶uble reducing substances and is, in fact (see later), the only sugar liberated during autolysis as determined by TLC and GLC analysis. The small difference between glucose solubilized and total reducing substances, calculated as glucose, is unaccounted for.

**Products of Strong Acid Hydrolysis of the Glucan.** The data of Figure 3 show the average of three replicate determinations of the time course of hydrolysis of the glucan in 1 M trifluoroacetic acid at 121 C. For this experiment, accurately weighed samples of about 2 mg of the glucan were suspended in acid, sealed in ampules at a slight negative pressure with N2 in the gas phase, and heated in an autoclave for the indicated time. As can be seen, 98% of the weight of the polymer can be accounted for as reducing sugar and 94% can be accounted for as glucose. Glucan weight was corrected for a 3% water content. Glucose was determined as reducing sugar (27) or as glucose by glucose oxidase (Glucostat, Worthington Biochemical Corporation) and its identity was confirmed by paper, TLC, and GLC analysis. Since the glucan absorbs moisture rapidly from the air it was necessary to obtain the dry weight of samples by extrapolation. For determination of the water content a sample of the polymer was accurately weighed on an electrobalance. The pan and wall samples were then dried for 24 hr in vacuo over anhydrous CaSO4 in an Abderhalden drying apparatus at the temperature of boiling 95% ethyl alcohol and dried a further 12 hr at room temperature over P2O5. The sample and pan were then weighed at 1-min intervals for 10 min and the initial dry weight was obtained by extrapolation to zero time. The water content of samples exposed to 50% humidity, room temperature air amounts to 2 to 3% after just 10 min.

**Absence of Monosaccharides Other than Glucose.** The glucan has also been hydrolyzed in 0.67 M HCl and in 3% (v/v) nitric acid with comparable quantitative results. The hydrolysis products obtained with trifluoroacetic acid, HCl, and HNO3 were examined by gas, thin layer, and paper chromatography and, in all cases, only glucose was detected. These data together with the quantitative analysis show that the polymer is a pure glucan. Since the walls are rich in pentosans and galactans,
polysaccharidase
eral
wall
autolysis
is
W-98.
Figure
4.
leads
or
laminaribiose,
and
several
tetrasaccharides
are
produced.
Disaccharides.
The
major
disaccharide
produced
by
acid
hydrolysis
is
cellobiose
and
it
accounts
for
about
90%
of
the
disaccharides
observed
by
TLC
and
GLC.
This
value
does
not,
of
course,
represent
abundance
since
disaccharide
hydrolysis
is
also
occurring.
Cellobiose
was
identified
by
its
chromato-
graphic
properties
on
paper
(Fig.
4)
and
on
TLC
(Table
II).
In
addition
the
α
and
β
anomers
were
detected
by
gas
liquid
chromatography
on
OV-1,
SE-30
(Table
I),
HIEFF
8-BP,
and
W-98.
Figure
4
illustrates
the
paper
chromatographic
patterns
obtained.
The
figure
also
demonstrates
that
laminaribiose
is
a
product
of
glucan
hydrolysis.
This
identification
was
con-
firmed
as
for
cellobiose.
Under
the
hydrolysis
conditions
employed,
laminaribiose
accounts
for
about
10%
of
the
disac-
charides
produced.
Gentiobiase
is
not
found
or
is
found
only
in
very
minute
amounts.
Isomaltose
and
other
disaccharides
are
detected.
Tri- and
Tetrasaccharides.
The
trisaccharide
3-O-β-cellobio-
syl-D-glucose
was
identified
as
a
product
of
glucan
hydrolysis.
The
chromatographic
behavior
of
this
compound
is
illustrated
in
the
data
of
Figure
4.
Its
identity
was
confirmed
by
TLC
(Table
II)
and
GLC
(Table
I)
and
by
a
comparison
of
the
mass
spectra
of
the	on
fragments
of
the
trimethylsilyl
ether.
The
occurrence
of
3-O-β-cellobiosyl-D-glucose
as
a
hydrolysis
product
demonstrates
that
the
glucan
contains
both
1→3
and
1→4
linkages
in
the
same
molecule.
At
least
two
other
trisac-
charides
and
two
tetrasaccharides
were
obtained
but
are
do
not
as
yet
characterized.
However,
as
is
shown
in
Figure
4,
the
olig-
saccharides
are
chromatographically
identical
to
those
obtained
by
hydrolysis
of
lichenan
and
oat
glucan
and
are
unlike
those
obtained
from
laminaran.

**Chromatographic
Profiles
of
Glucan
Hydrolysis Products.**
Examination
of
the
paper
chromatographic
patterns
of
the
acid
hydrolysis
products
of
the
glucan,
lichenan,
oat
glucan,
and
laminaran
is
illustrated
by
the
data
of
Figure
4.
The
cell
wall
glucan,
lichenan,
and
oat
glucan
yield
similar
profiles.
All
show
cellobiose
as
the
major
disaccharide
with
smaller
amounts
of
laminaribiose.
Laminaran
does
not
yield
cellobiose
but
yields
instead
the
series
laminaribiose,
laminaritriose,
and
laminaritetrose.
Glucan,
lichenan,
and
oat
glucan
do
not
yield
laminaritriose,
indicating
the
absence
of
a
series
of
1→3
linked
sugars.
Glucan,
lichenan,
and
oat
glucan
all
yield
3-O-β-cel-
lobiosyl-D-glucose,
showing
that
1→3
and
1→4
linkages
occur
in
the
same
linear
glucan
chain.
Under
the
hydrolysis
con-
ditions
employed
none
of
the
polysaccharides
yielded
gentiobiase.
This
is
expected
for
lichenan
and
oat
glucan
as
these
are
bel-
ieved
not
to
contain
1→6
linkages
(31,32).
It
can
further
be
seen
that
the
uncharacterized
but
reasonably
well
resolved
tri-
and
tetrasaccharides
from
the
cell
wall
glucan
appear
to
be
identical
to
those
obtained
from
lichenan
and
oat
glucan.
Thus,
the
cell
wall
glucan
is
essentially
identical
to
lichenan
and
oat
glucan
in
terms
of
acid
hydrolysis
products.

**Enzymatic
Hydrolysis.**
A
limited
study
has
been
made
of
the
susceptibility
of
the
glucan
to
hydrolysis
by
polysacchari-
dases.
The
glucan
is
not
attacked
by
a
fungal
exo-β-(1→3)

Fig. 4.
Paper
chromatographic
comparison
of
the
products
of
weak
acid
hydrolysis
of
the
polysaccharides
laminaran,
lichenan,
oat
glucan,
and
cell
wall
glucan.
Chromatography
was
by
the
des-
ceding
method
on
Whatman
No.
1
paper
for
120
hr.
Approx-
imately
200
µg
of
carbohydrate
were
used
for
each
original
spot.
Conditions
of
chromatography
and
detection
of
sugars
were
as
in-
dicated
in
the
text. 3-O-β-C: 3-O-β-cellobiosyl-D-glucose; LTR:
laminaritriose;
GB:
gentiobiase;
CB:
cellobiose;
LB:
laminaribiose.
Migrations
are
expressed
relative
to
that
of
laminaribiose,
which
had
moved
about
30
cm.

DISCUSSION

Our
present
knowledge
of
the
chemistry
of
the
primary
cell
cells
does
not
adequate
to
decide
which
component(s)
imparts
structural
rigidity
to
the
wall.
Thus,
“wall
pressure,”
with
its
intended
implications
for
growth,
remains
a
physical
concept
and,
owing
to
the
complexity
of
the
wall,
a
direct
structural
approach
is
difficult.
In
this
work
and
in
preceding
studies
in
this
series,
we
have
chosen
instead
to
attempt
to
determine
what
wall-bound
enzymes
can
do
to
the
cell
wall
(16,17).
Hopefully,
such
studies
may
lead
to
an
understanding
of
the
metabolic
control
of
cell
rigidity.
We
find
that
wall
enzymes of cell walls of corn coleoptiles solubilize a glucan from the wall and we present a partial chemical characterization of the glucan.

Previous workers, using different approaches, have added data showing that wall polysaccharides—and particularly glucosyl- and galactosyl-polysaccharides—are metabolically increased and decreased in amount during growth (2, 20, 24, 25, 29).

Of particular interest for this study are the reports that in *Avena* coleoptile sections (9, 34), *Pisum* sections (19), and bean seedlings (28, 29) acid-soluble noncellulosic glucose polymers decrease in amount during extension growth. Further, there are reports that glucanase may play a role in auxin-induced extension growth (21, 23, 39, 41, but see 22 and 36) and that walls from rapidly growing tissues have more glucan (5, 7, 26, 28) or cellulase (6) activity. There are also reports that β-glucosidase activity is located primarily on the cell walls (4) or the cell surface (11) and, most importantly, that call-labeled cell wall polysaccharides can be hydrolyzed by cell wall preparations to an oligosaccharide and then to free glucose (10). We feel that it is established that a call wall glucan is hydrolyzed by a cell wall-bound glucanase during extension growth. Our data provide insight as to the structure of this glucan.

There are, to our knowledge, no previously published partial chemical characterizations of a purified noncellulosic glucan from primary cell walls. It thus is of interest to find that the wall glucan is of the lichenan type. It resembles lichenan, the poly-β-D-glucan from Iceland moss (3, 32), in that it is a glucan and yields the same partial acid hydrolysis products as does lichenan (32). Most importantly, it yields 3-O-β-cellubiosyl-D-glucose, thus demonstrating that the wall glucan, like lichenan, contains both 1→3 and 1→4 linkages. Katz, cited by Ordin and Hall (30), observed a trisaccharide produced by cellulase treatment of HCl extracts of *Avena* wall which Ordin and Hall found to be laminarinbiosyl glucose, a mixed 1→3, 1→4-oligosaccharide. This earlier observation, together with the present findings, may indicate a more general distribution of lichenan-like polymers. Interestingly, Karrr and coworkers (cited by Morris, [25]) had originally suggested the name “reserve cellulose” for lichenan-like polymers. This was based upon their belief in the ubiquitous distribution of lichenan since lichenan was common in seeds. However, this report and the prior ones of the occurrence of a lichenan-like polymer in oat seeds (25) and in barley (31) are the only ones of which we have knowledge. It will be of interest to determine if lichenan-like glucans are more common in nature.

Acknowledgments—We wish to thank Professor C. C. Sweeney for making available the mass spectrometric facility and Mr. Jack E. Harten for his assistance in the mass spectrometric analysis. We are indebted to Dr. E. T. Reese and Professor W. Z. Hasid for valuable carbohydrate standards and to Dr. John Dever for some of the earlier studies of hydrolysis products.

**LITERATURE CITED**